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journal or publication title	東北大学浅虫臨海実験所報告
volume	18
number	3
page range	103-108
year	1989-03-25
URL	http://hdl.handle.net/10097/00131477

STUDY OF SEWAGE SLUDGE TREATMENT BY MEIOBENTHOS,
NITOCRA SP. AND MACROBENTHOS,
NEANTHES JAPONICA (IZUKA)

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The role of a meiobenthic Harpacticoida, *Nitocra* sp., on removal of particulate organic matter in an artificial tidal flat used for culturing the polychaete *Neanthes japonica* (Izuka) was examined. *Nitocra* sp. was able to ingest a large quantity of sludge extracted from sewage waste and feces excreted from *N. japonica* fed on sludge, and completed one life cycle within three weeks. On the other hand, *N. japonica* could not predate *Nitocra* sp. at any stage during its life cycle. These results suggest that the co-existence of *Nitocra* sp. and *N. japonica* is suited for the removal of particulate organic matter in an artificial tidal flat.

A pilot plant with an artificial tidal flat simulating natural estuarine tidal flat conditions was constructed and operated as a habitat for the polychaete, *Neanthes japonica* (Izuka), and it was shown that the sludge extracted from a domestic sewage treatment plant, mainly bacteria and protozoa, serves as food for the polychaete (KURIHARA, 1983). Thus an artificial tidal flat may be considered as a method for sludge treatment, thus helping to solve a serious problem in modern urban management.

It has been reported by several investigators that the feces of macrobenthos is an important food source for meiobenthos (MARE, 1942; NEWELL, 1965; JOHANNES and SATOMI, 1966; FRANKENBERG and SMITH JR., 1967; FRANKENBERG *et al.*, 1967; FENCHEL, 1969; RHOADS, 1974). Therefore the introduction of coprophagous meiobenthos into an artificial tidal flat is considered to be useful for treating the sludge, because a considerable fraction of the feces of *N. japonica* may be eliminated by the feeding activity of the meiobenthos.

The purpose of the present study was to find out whether coprophagous meiobenthos can adapt to the brackish tidal flat and to elucidate its role in trophic relations there. Vertical distribution and abundance were investigated initially in the tidal flat where *N. japonica* occurs densely, since *N. japonica* excretes its feces on the surface around the burrow entrance (TSUCHIYA and KURIHARA, 1979, 1980). Subsequently sewage sludge, *N. japonica* feces and their own feces were all given to

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the dominant meiobenthos as food sources, and growth of the meiobenthos, mortality and sex ratio were then investigated. In addition, the predator-prey relationship was examined between *N. japonica* and the meiobenthos.

MATERIALS AND METHODS

The field survey was made at a tidal flat of Gamô Lagoon, Miyagi Prefecture, in northeastern Japan (38°15'N.; 141°01'E.). The tidal flat was submerged with brackish water twice a day, and *N. japonica* occurred there densely (TSUCHIYA and KURIHARA, 1976). The vertical distribution and abundance of meiobenthos were investigated on 17 September 1979. Core samplers 3 cm in diameter and 10 cm in length were inserted randomly into the substratum at low tide, and samples were collected without disturbing the vertical structure. Meiobenthos from a depth of 0 (surface) to 8 cm was isolated from the mud by the flotation method of Iro (1970) and then identified and counted.

The food source for the meiobenthos i.e. *Nitocra* sp., which dominated the substratum near the surface, was prepared. The sludge was collected from sewage treatment plants, and passed through a sieve of 2-mm mesh. The sludge was added to brackish water (salinity=13‰), passed through a glass filter (Toyo Kagaku Co., Ltd.), and subsequently mixed. The mixture was centrifuged at 4000 rpm for 20 min, and divided into a liquid fraction containing soluble matter and a solid fraction (subsequently referred to as "sludge"). The above treatment was repeated three times.

The fecal pellets from *N. japonica* previously fed on "sludge" were collected by pipetting, and then washed by shaking gently in brackish water (salinity=13‰). The fecal pellets from adult *Nitocra* sp. fed on feces of *N. japonica* were also collected by pipetting and washed in brackish water.

1st-stage nauplius larvae of *Nitocra* sp. hatched out from cultured ovigerous females were collected in order to synchronize the growth process. Appropriate amounts of "sludge", *N. japonica* feces and *Nitocra* sp. feces were put in three Petri dishes (diameter=6 cm) filled with brackish water (salinity=13‰), respectively. Subsequently, 45, 161 or 27 individuals of 1st-stage nauplius larvae of *Nitocra* sp. were added to the three Petri dishes, each containing one of the three kinds of food sources. The growth process of the nauplii was observed in each dish using a stereoscopic microscope, and the number of individuals counted. The cultivations were carried out in a room at a constant temperature of $20 \pm 2^\circ\text{C}$ in continuous darkness.

Since the shape of the fecal pellet is similar to an ellipsoid, the volume for each stage of the growth process was calculated from the pellet width and length.

The predator-prey relationship was examined between *N. japonica* and *Nitocra* sp. Some individuals of *N. japonica* were put in a tall beaker (volume=300 ml)

with sea sand (100 ml) and filtered brackish water (salinity=13‰), and then fasted for one day. 82 individuals of *Nitocra* sp. were then added to the tall beaker containing *N. japonica*. After two days, the number of individuals of *Nitocra* sp. was counted.

RESULTS

The vertical distribution and abundance of meiobenthos in the substratum of the tidal flat are shown in Fig. 1. Harpacticoida (*Nitocra* sp.), Nematoda, Oligochaeta and Ostracoda were observed. *Nitocra* sp. dominated near the surface i.e. from 0 to 2 cm. The number of individuals of *Nitocra* sp. decreased in the substratum below 2 cm. These results suggest that *Nitocra* sp. feeds mainly upon organic matter on the surface of the substratum, e.g., deposited matter and feces of macrobenthos such as *N. japonica*.

Nitocra sp. has six nauplius stages and six copepodid stages. Figure 2 shows the growth process of *Nitocra* sp., when "sludge" or *N. japonica* feces was given to new-born nauplii as the food source. New-born nauplii grew into the 1st copepodid stage within 9 or 7 days, and developed to the 6th copepodid stage (adult) within 15 or 14 days, when fed on "sludge" or *N. japonica* feces, respectively. Ovigerous

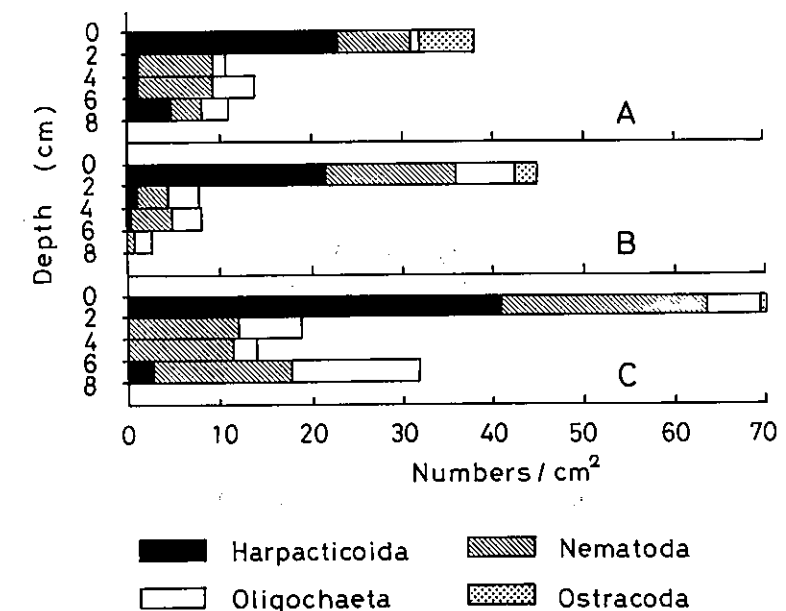


Fig. 1. Vertical distribution of some meiobenthic invertebrates in the tidal flat of Gamô lagoon. "A" indicates the sampling point at the level of mean low water at spring tide, "B" indicates the mean tidal level, and "C" indicates the level of mean high water at spring tide.

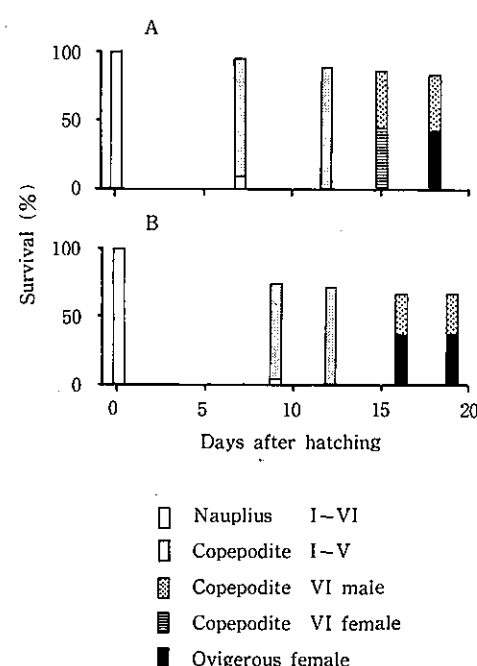


Fig. 2. Growth processes and survival rates of *Nitocra* sp. during one life cycle, when the feces of *N. japonica* (A) or sludge (B) were given to 1st-stage nauplius larvae, respectively.

females were observed after 16 or 15 days, and nauplii of the next generation appeared after 20 or 18 days. The mortality during the period from the new-born nauplius to adult stage was 32% when fed on "sludge", or 22% on *N. japonica* feces. The male ratio at the adult copepoda stage was 48.4% when fed on "sludge" or 54.3% on *N. japonica* feces. The mortality, sex ratio, and growth process did not differ appreciably between the populations fed on "sludge" or *N. japonica* feces. These results indicate that *N. japonica* feces as well as "sludge" contained sufficient nutritive value for development of *Nitocra* sp. Although new-born nauplius larvae ingested the fecal pellets of adults of *Nitocra* sp. and subsequently defecated, they

Table 1.
Feces volume at each developmental stage of *Nitocra* sp. "M" indicates male, and "F" indicates female, at the copepodite VI stage.

Feces volume ($\times 10^{-4}$ mm ³)											
Nauplius						Copepodite					
I	II	III	IV	V	VI	I	II	III	IV	V	VI
											(M) (F)
0.024	0.052	0.096	0.13	0.19	0.24	0.26	0.26	0.33	0.61	1.0	1.0 1.8

did not grow.

Table 1 shows the fecal pellet volume for each developmental stage of *Nitocra* sp. The length of the fecal pellet at the nauplius stage was 20 to 60 μ m, and that at the copepodid stage ranged from 160 to 200 μ m. The volume of the fecal pellet of the 1st nauplius-stage larva was 0.024×10^{-4} mm³ and that of the adult female was 1.8×10^{-4} mm³, 75 times more than the 1st nauplius-stage larva.

When *Nitocra* sp. was cultured together with hungry *N. japonica* for two days, 91.5% (75 individuals) of the former was survived.

DISCUSSION

Nitocra sp. fed on the feces of *N. japonica* which had itself been fed on "sludge" was able to complete one life cycle (Fig. 2), indicating that there was sufficient nutritive value for *Nitocra* sp. Although *N. japonica* fed on matter at the surface (TSUCHIYA and KURIHARA, 1979, 1980), a large number (91.5%) of individuals of *Nitocra* sp. survived, when *Nitocra* sp. was cultured together with hungry *N. japonica* in a tall beaker. It has been reported that *N. japonica* feeds on deposited particles less than 37 μ m in diameter (TSUCHIYA & KURIHARA, 1979). The body length of *Nitocra* sp. was about 80 μ m at the 1st nauplius-stage and 550 μ m for the adult female, suggesting that this organism is too big for *N. japonica* to swallow. Therefore, *Nitocra* sp. co-exists with *N. japonica* throughout its life cycle in the artificial tidal flat suitable as a habitat for *N. japonica*.

The volume of feces of *Nitocra* sp. (Table 1) was smaller than that of *N. japonica* (length=about 2000 μ m), indicating that *Nitocra* sp. breaks down the feces of *N. japonica* to smaller particles by its feeding activity. In other words, the ratio of surface area to volume of the fecal pellet increases when the feces of *N. japonica* pass through the gut of *Nitocra* sp.

Only colourless flagellates were observed on the feces of *N. japonica*, while a relatively wide range of microbes, colourless flagellates, Hypotrichida and Trichostomatida, were recognized on the feces of *Nitocra* sp. These observations suggest that the feces of *Nitocra* sp. are attacked by more species of microbe. FENCHEL (1970) has reported on the positive relationship between area of detritus and number of microbial individuals, and ODUM and CRUZ (1967) indicated that the activity of microbial decomposition increases with decrease in the particle size of detritus. The fecal pellets of *Nitocra* sp. lost their original shape in the laboratory faster than did those of *N. japonica*. These results suggest that the feces of *Nitocra* sp. are decomposed more easily than those of *N. japonica*.

These various observations all suggest that the mineralization of sludge is accelerated by the respiration of *N. japonica*, when it feeds upon the sludge, and of *Nitocra* sp. when fed on the feces of *N. japonica* itself fed on sludge, under aerobic environmental conditions in an artificial tidal flat.

The authors wish to express their cordial thanks to Dr. S. TAKEDA, Faculty of Science of Tôhoku University, for critically reviewing the manuscript. This study was partially supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture of Japan (No. 63115019).

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